

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

Claims 1-200 (cancelled):

201. (new) A method of expanding an *ex-vivo* population of hematopoietic stem cells, while at the same time, substantially inhibiting differentiation of the hematopoietic stem cells *ex-vivo*, the method comprising

providing hematopoietic mononuclear cells;

culturing said mononuclear cells *ex-vivo* under conditions allowing for cell proliferation and, at the same time, culturing said cells under conditions selected from the group consisting of

conditions reducing expression and/or activity of CD38 in said mononuclear cells,

conditions reducing capacity of said hematopoietic mononuclear cells in responding to retinoic acid, retinoids and/or Vitamin D in said mononuclear cells,

conditions reducing capacity of said hematopoietic mononuclear cells in responding to signaling pathways involving the retinoic acid receptor, the retinoid X receptor and/or the Vitamin D receptor in said mononuclear cells;

culturing said mononuclear cells in the presence of nicotinamide, a nicotinamide analog, a nicotinamide or a nicotinamide analog derivative or a nicotinamide or a nicotinamide analog metabolite in said mononuclear cells;

conditions reducing an expression and/or activity of PI 3-kinase in said mononuclear cells; and

culturing said mononuclear cells in the presence of at least one copper chelator or chelate,

thereby expanding a population of said hematopoietic stem cells while at the same time substantially inhibiting differentiation of said hematopoietic stem cells *ex-vivo*.

202. (new) A method of transplanting or implanting hematopoietic cells, the method comprising:

(a) obtaining hematopoietic mononuclear cells;

(b) culturing said mononuclear cells *ex vivo* for cell proliferation, wherein said culturing is performed in a condition selected from the group consisting of

reducing expression and/or activity of CD38,

- reducing a capacity of said hematopoietic mononuclear cells in responding to retinoic acid, retinoids and/or Vitamin D;
  - reducing capacity of said hematopoietic mononuclear cells in responding to signaling pathways involving the retinoic acid receptor, the retinoid X receptor and/or the Vitamin D receptor;
  - the presence of nicotinamide, a nicotinamide analog, a nicotinamide or a nicotinamide analog derivative or a nicotinamide or a nicotinamide analog metabolite;
  - reducing an expression and/or activity of PI 3-kinase or
  - the presence of at least one copper chelator or chelate,
- thereby expanding a population of said hematopoietic stem cells, while at the same time, substantially inhibiting differentiation of said hematopoietic stem cells *ex-vivo*; and
- (c) transplanting or implanting said hematopoietic stem cells to a recipient.

203. (new) The method of claim 202, wherein said donor and said recipient are a single individual.

204. (new) A method of genetically modifying hematopoietic stem cells with an exogene comprising:

- (a) obtaining hematopoietic mononuclear cells;
- (b) culturing said mononuclear cells *ex vivo* for cell proliferation, wherein said culturing is performed in a condition selected from the group consisting of
  - conditions reducing expression and/or activity of CD38 in said mononuclear cells,
  - conditions reducing capacity of said hematopoietic mononuclear cells in responding to retinoic acid, retinoids and/or Vitamin D in said mononuclear cells,
  - conditions reducing capacity of said hematopoietic mononuclear cells in responding to signaling pathways involving the retinoic acid receptor, the retinoid X receptor and/or the Vitamin D receptor in said mononuclear cells;
  - culturing said mononuclear cells in the presence of nicotinamide, a nicotinamide analog, a nicotinamide or a nicotinamide analog derivative or a nicotinamide or a nicotinamide analog metabolite in said mononuclear cells;

conditions reducing an expression and/or activity of PI 3-kinase in said mononuclear cells; and

culturing said mononuclear cells in the presence of at least one copper chelator or chelate,

thereby expanding a population of said hematopoietic stem cells, while at the same time, substantially inhibiting differentiation of said hematopoietic stem cells *ex-vivo*; and

(c) genetically modifying said hematopoietic stem cells with the exogene.

205. (new) The method of claim 204, wherein genetically modifying is effected by a vector which comprises the exogene.

206. (new) The method of claim 205, wherein the vector is a viral vector or a nucleic acid vector.

207. (new) A method of adoptive immunotherapy comprising:

(a) obtaining hematopoietic mononuclear cells from a recipient;

(b) culturing said mononuclear cells *ex vivo* for cell proliferation, wherein said culturing is performed in a condition selected from the group consisting of

- conditions reducing expression and/or activity of CD38 in said mononuclear cells,
- conditions reducing capacity of said hematopoietic mononuclear cells in responding to retinoic acid, retinoids and/or Vitamin D in said mononuclear cells,
- conditions reducing capacity of said hematopoietic mononuclear cells in responding to signaling pathways involving the retinoic acid receptor, the retinoid X receptor and/or the Vitamin D receptor in said mononuclear cells;

culturing said mononuclear cells in the presence of nicotinamide, a nicotinamide analog, a nicotinamide or a nicotinamide analog derivative or a nicotinamide or a nicotinamide analog metabolite in said mononuclear cells;

conditions reducing an expression and/or activity of PI 3-kinase in said mononuclear cells; and

culturing said mononuclear cells in the presence of at least one copper chelator or chelate, thereby expanding a population of said hematopoietic stem cells, while at the same time, substantially inhibiting differentiation of said hematopoietic stem cells; and

(c) transplanting said hematopoietic stem cells to the recipient.

208. (new) A transplantable hematopoietic cell preparation comprising an expanded population of hematopoietic stem cells propagated *ex-vivo* from hematopoietic mononuclear cells in the presence of an effective amount of an agent,

wherein said agent has an activity selected from the group consisting of

reducing expression and/or activity of CD38 in said mononuclear cells,

reducing capacity of said hematopoietic mononuclear cells in responding to retinoic acid, retinoids and/or Vitamin D in said mononuclear cells,

reducing capacity of said hematopoietic mononuclear cells in responding to signaling pathways involving the retinoic acid receptor, the retinoid X receptor and/or the Vitamin D receptor in said mononuclear cells; and

reducing an expression and/or activity of PI 3-kinase in said mononuclear cells; or wherein said agent is

a copper chelator or chelate, or

nicotinamide, a nicotinamide analog, a nicotinamide or a nicotinamide analog derivative or a nicotinamide or a nicotinamide analog metabolite in said mononuclear cells;

while at the same time, substantially inhibiting differentiation of said hematopoietic stem cells, and a pharmaceutically acceptable carrier.

209. (new) The method of claim 201, wherein said hematopoietic mononuclear cells are derived from a source selected from the group consisting of bone marrow, peripheral blood and neonatal umbilical cord blood.

210. (new) The method of claim 201, wherein providing said hematopoietic mononuclear cells with said conditions for *ex-vivo* cell proliferation comprises providing said hematopoietic mononuclear cells with nutrients and with cytokines.

211. (new) The method of claim 210, wherein said cytokines are early acting cytokines.

212. (new) The method of claim 211, wherein said early acting cytokines are selected from the group consisting of stem cell factor, FLT3 ligand, interleukin-1, interleukin-2, interleukin-3, interleukin-6, interleukin-10, interleukin-12, tumor necrosis factor- $\alpha$  and thrombopoietin.

213. (new) The method of claim 210, wherein said cytokines are late acting cytokines.

214. (new) The method of claim 213, wherein said late acting cytokines are selected from the group consisting of granulocyte colony stimulating factor, granulocyte/macrophage colony stimulating factor, erythropoietin, FGF, EGF, NGF, VEGF, LIF, Hepatocyte growth factor and macrophage colony stimulating factor.

215. (new) The method of claim 201, wherein providing said hematopoietic mononuclear cells with *ex-vivo* culture conditions for reducing said expression and/or said activity of CD38 is by providing said hematopoietic mononuclear cells with an agent that downregulates CD38 expression.

216. (new) The transplantable hematopoietic cell preparation of claim 208, wherein said agent is an agent that downregulates CD38 expression.

217. (new) The method of claim 215, wherein the agent that downregulates CD38 expression is selected from the group consisting of a retinoic acid receptor antagonist, a retinoid X receptor antagonist and a Vitamin D receptor antagonist.

218. (new) The method of claim 215, wherein the agent that downregulates CD38 expression is an antagonist for reducing a capacity of said hematopoietic mononuclear cells in responding to retinoic acid, retinoid and/or Vitamin D.

219. (new) The method of claim 215, wherein said agent that downregulates CD38 expression is a polynucleotide.

220. (new) The method of claim 219, wherein the polynucleotide encodes an anti CD38, an anti retinoic acid receptor, an anti retinoid X receptor or an anti Vitamin D receptor intracellular antibody.

221. (new) The method of claim 219, wherein the polynucleotide encodes an anti CD38, an anti retinoic acid receptor, an anti retinoid X receptor or an anti Vitamin D receptor antibody.

222. (new) The method of claim 219, wherein said polynucleotide is a small interfering polynucleotide molecule directed to cause intracellular CD38, retinoic acid receptor, retinoid X receptor or Vitamin D receptor mRNA degradation.

223. (new) The method of claim 222, wherein said small interfering polynucleotide molecule is selected from the group consisting of an RNAi molecule, an anti-sense molecule, a rybozyme molecule and a DNAzyme molecule.

224. (new) The method of claim 215, wherein said agent that downregulates CD38 expression is an agent that downregulates PI 3-kinase expression.

225. (new) The method of claim 224, wherein said agent that downregulates PI 3-kinase expression is a polynucleotide.

226. (new) The method of claim 224, wherein agent that downregulates PI 3-kinase expression is an intracellular antibody.

227. (new) The method of claim 225, wherein said polynucleotide is a small interfering polynucleotide molecule directed to cause intracellular PI 3-kinase mRNA or gene degradation.

228. (new) The method of claim 227, wherein said small interfering polynucleotide molecule is selected from the group consisting of an RNAi molecule, an anti-sense molecule, a rybozyme molecule and a DNAzyme molecule.

229. (new) The method of claim 215, wherein said agent that downregulates CD38 expression is an agent that inhibits PI 3-kinase activity.

230. (new) The method of claim 229, wherein said agent that inhibits PI 3-kinase activity is selected from the group consisting of wortmannin and LY294002

231. (new) The method of claim 201, wherein providing said hematopoietic mononuclear cells with *ex-vivo* culture conditions for reducing said expression and/or said activity of CD38 is by providing said hematopoietic mononuclear cells with an agent that inhibits CD38 activity.

232. (new) The transplantable hematopoietic cell preparation of claim 208, wherein said agent is an agent that inhibits CD38 activity.

233. (new) The method of claim 232, wherein said agent that inhibits CD38 activity is nicotinamide, a nicotinamide analog, a nicotinamide or a nicotinamide analog derivative or a nicotinamide or a nicotinamide analog metabolite.

234. (new) The method of claim 233, wherein said nicotinamide analog is selected from the group consisting of benzamide, nicotinthioamide, nicotinic acid and  $\alpha$ -amino-3-indolepropionic acid.

235. (new) The method of claim 201, wherein providing said hematopoietic mononuclear cells with *ex-vivo* culture conditions for reducing said expression and/or said activity of CD38 is by providing said hematopoietic mononuclear cells with an agent that inhibits PI 3-kinase activity.

236. (new) The transplantable hematopoietic cell preparation of claim 208, wherein said agent is an agent that inhibits PI 3-kinase activity.

237. (new) The method of claim 236, wherein said agent that inhibits PI 3-kinase activity is selected from the group consisting of wortmannin and LY294002.

238. (new) The method of claim 201, wherein said hematopoietic mononuclear cells are not enriched prior to culturing *ex-vivo* under conditions allowing for cell proliferation.

239. (new) The method of claim 201, wherein said hematopoietic cells comprise a major fraction of hematopoietic committed cells and a minor fraction of hematopoietic stem and progenitor cells.

240. (new) An assay for determining whether a transition metal chelate or chelator causes substantial inhibition or induction of differentiation of hematopoietic stem cells, the assay comprising:

culturing hematopoietic mononuclear cells in the presence of the transition metal chelate or chelator and monitoring differentiation of said hematopoietic stem cells, wherein if differentiation is increased as is compared to non-treated hematopoietic mononuclear cells, said transition metal chelate induces differentiation, whereas if differentiation is decreased as is compared to non-treated hematopoietic mononuclear cells, or if differentiation is absent altogether, said transition metal chelate inhibits differentiation.

241. (new) An assay for identifying an effective hematopoietic stem cell expansion agent, the assay comprising culturing hematopoietic mononuclear cells in the presence of a compound selected from the group consisting of

a retinoic acid receptor antagonist;  
retinoid X receptor antagonist;  
vitamin D receptor antagonist;  
agent that inhibits PI 3-kinase activity; and  
a nicotinamide analog, a nicotinamide or a nicotinamide analog derivative or a nicotinamide or a nicotinamide analog metabolite,

and monitoring expansion of said hematopoietic stem cells, wherein if increased expansion and decreased differentiation of said hematopoietic stem cells occurs, as compared to non-treated hematopoietic mononuclear cells, the compound is an effective hematopoietic stem cell expansion agent.



242. (new) A hematopoietic stem cells collection/culturing bag supplemented with an effective amount of a compound selected from the group consisting of

a retinoic acid receptor antagonist, a retinoid X receptor antagonist and/or a Vitamin D receptor antagonist,

nicotinamide, a nicotinamide analog, a nicotinamide or a nicotinamide analog derivative or a nicotinamide or a nicotinamide analog metabolite; or

an agent that inhibits PI 3-kinase activity,

which substantially inhibits cell differentiation of a hematopoietic stem cells fraction of hematopoietic mononuclear cells.

243. (new) An *ex-vivo* expanded population of hematopoietic stem cells, obtained by the method of claim 1.

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